

Assessment of *Allium Sativum*, *Zingiber Officinale* Extracts on *Plasmodium Berghei* Infected Male Wistar Rats

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ABSTRACT

The aim of the study was to investigate the separate and combined effects of *Allium sativum* (garlic) and *Zingiber officinale* (ginger) on the parasite levels, liver enzymes, histology and haematological parameters. Forty-two (42) male wistar rats were assigned into seven (7) groups as mentioned below: Group 1: served as negative control group and received distilled water, Group 2: served as positive control group and received 0.2ml PB (I.P single dose), Group 3: received 0.2ml PB (I.P single dose) + 500 mg/kg of allium sativum; Group 4: received 0.2ml PB (I.P single dose) + 500 mg/kg of zingiber officinale; Group 5: received 0.2ml PB (I.P single dose) + 250 mg/kg of allium sativum + 250 mg/kg of zingiber officinale; Group 6: received 0.2ml PB (I.P single dose) + artemether/Lumefantrine 80/480mg (4mg/kg and 8mg/kg); Group 7: received 0.2ml PB (I.P single dose) + Dihydroartemisinin and piperaquine 30mg/225mg (2mg/kg and 18mg/kg). All administrations were given orally twice daily for a period of 4 days, after 3 days infection period. At the end of the treatment, three (3) male wistar rats per group were sacrificed for the first test. After, 7 days post treatment the second test was done, three (3) male wistar rats per group; samples were collected for determination of hematological analysis, parasitemia levels, liver enzymes, and liver histology. Results represented as mean \pm SEM were compared using One-Way and Repeated measure ANOVA. It was observed that higher percentage of malaria parasitaemia seen in parasitized and untreated wistar rats were reduced in parasitized and treated groups. *Allium sativum* and *Zingiber officinale* extracts possesses no lethality in rats at 6000mg/kg body weight. Overall, the results suggest that administration of the different extracts were effective in parasite clearance and none toxic.

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KEYWORDS: *Plasmodium Berghei*, *Allium sativum*, *Zingiber officinale*, liver enzymes

INTRODUCTION

Malaria remains a life-threatening infectious disease in the world today, with a very high morbidity and mortality rate annually and it is caused by the plasmodium parasites, transmitted through the bites of an infected female anopheles mosquito (WHO, 2022). Malaria is preventable, treatable and curable, it is endemic in Nigeria and has remain a big public health concern in Nigeria, affecting mostly children below five years of age and pregnant women (NPC, 2019).

There are five plasmodium species in human but most cases of malaria and death are caused by plasmodium

falciparum or plasmodium vivax (Esan *et al.*, 2014; WHO, 2022). However, for malaria studies using rodents, the rodent parasite plasmodium berghe is generally adopted due to its common characteristics with the human parasite plasmodium falciparum (Ogbueli *et al.*, 2014).

An estimated 241 million cases of malaria with 627 000 deaths was recorded globally in the year 2020. Africa carries a disproportionate proportion of the global burden, 95% cases and 96% deaths of the global burden were recorded in Africa with 80% of the deaths in children below five years (WHO, 2022).

Four Africa nations accounted for just over half of the global malaria death cases: Nigeria (31.9%), the Democratic Republic of the Congo (13.2%), United Republic of Tanzania (4.1%) and Mozambique (3.8%) (WHO, 2022).

To reduce the malaria burden globally, the World Health Organization (WHO) recommended the use of Artemisinin Combination Therapy (ACT), insecticide-treated nets (ITNs), indoor residual spraying (IRS) and vaccines. While vaccines were recently approved for children, 39% of Nigerians still don't use ITS, availability and unaffordability of medications as well as the malaria parasites developing resistance to the available medication has fueled the source for alternative therapy in plants (WHO, 2018; NPC, 2019; WHO, 2022)

The use of herbs for medicinal purposes is as old as mankind especially in Africa (Abd El-Ghani, 2016). Most samples containing alkaloid are used in Nigeria for the treatment of malaria and fever, (Adesuga and Cooker, 2001). Some of these plants include garlic (*Allium Sativum*) and ginger (*Zingiber officinale*). Ginger and Garlic are both local herbs widely used in Africa as supplements in food and for various medical purposes (Block, 2010). All of these and the desire for low-cost treatment have led to a series of research to decipher alternative therapy for malaria (Weathers *et al.*, 2014)

STUDY AREA

This study was conducted in the department of human physiology, faculty of basic sciences, Nnamdi Azikiwe University, Nnewi campus Okofia, Anambra state, Nigeria.

EXPERIMENTAL ANIMALS

In this study forty- two (42) adult male wistar rats weighing between 150-180g were recruited for this study. The animals were kept in the animal house of

the Department of Human Physiology, Nnamdi Azikiwe University, Nnewi Campus. They were allowed to acclimatize to the laboratory environment for a period of two weeks before the commencement of the experiment. The rats were given free access to feed and drinking water *ad libitum* during the experimental period. Ethical approval consent was obtained for the progress of this study from the Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus. Rats handling and treatments conform to guidelines of the Nnamdi Azikiwe University Animal Research Ethics Committee (NAU-AREC) for laboratory animal care and use.

EXPERIMENTAL DESIGN

Forty-two (42) male wistar rats were assigned into seven (7) groups (6 rats/group) as mentioned below: Group 1: served as the negative control group and received 1 ml/kg of distilled water, Group 2: served as the positive control group and received 0.2ml PB (single dose), Group 3: received 0.2ml PB (single dose) + 500 mg/kg of *allium sativum*; Group 4: received 0.2ml PB (single dose) + 500 mg/kg of *zingiber officinale*; Group 5: received 0.2ml PB (single dose) + 250 mg/kg of *allium sativum* + 250 mg/kg of *zingiber officinale*; Group 6: received 0.2ml PB (single dose) + artemether/Lumefantrine 80/480mg (4mg/kg and 8mg/kg); Group 7: received 0.2ml PB (single dose) + Dihydroartemisinin and piperazine 30mg/225mg (2mg/kg and 18mg/kg). All administrations were given orally twice daily for a period of 3 days. At the end of the treatment, three (3) male wistar rats per group were sacrificed. Finally, fourteen (14) days post treatments the final sacrifice was done, three (3) male wistar rats per group; samples were collected for determination of hematological analysis, parasitemia levels, liver enzymes, and liver histology.

TABLE 1: ANIMAL GROUPING

Group	No. Of Animals	Parasitization And Treatment
I	6	distilled water and rat pellets
II	6	0.2ml BP (I.P single dose)
III	6	0.2ml PB (I.P single dose) + <i>allium sativum</i> extracts 500mg/kg
IV	6	0.2ml PB (I.P single dose) + <i>zingiber officinal</i> extracts 500mg/kg
V	6	0.2ml PB (I.P single dose) + <i>zingiber officinal</i> 250mg/kg + <i>allium sativum</i> extracts 250mg/kg
VI	6	0.2ml PB (I.P single dose) + artemether/Lumefantrine 80/480mg (10mg/kg)
VII	6	0.2ml PB (I.P single dose) + Dihydroartemisinin and piperazine 30mg/225mg (2mg/kg and 18mg/kg)

I.P = Intra-peritoneally

P.B = Plasmodium Berghei

COLLECTION AND PREPARATION OF PLANT EXTRACTS

Fresh plants of ginger rhizomes and garlic bulbs were purchased from the local market in (Eke Amobi) Otolu Nnewi, Nnewi North L.G.A of Anambra state. Ginger rhizomes and garlic bulbs were washed, peeled, and air-

dried under ambient temperature. The dried ginger and garlic were separately ground using a sterile electric blender to a fine powder.

EXTRACTION PROCEDURES

Extracts were prepared as previously reported in literature (Bhandari *et al.*, 2005). 250g of the powdered ginger and 250g of garlic was soaked in 1000ml of 98% absolute ethanol separately and was allowed for 48 hours at room temperature in a beaker after which it was sieved using porcelain cloth and was filtered using No. 1 filter paper into a clean glass beaker. The filtrate was concentrated using a digital rotatory evaporator (TT-52 Technel and Technel USA) and was dried using a thermostat oven (DHG- 9023A PEC medicals USA) into a gel-like substance and stored in a refrigerator (Nexus).

PARASITE INOCULATION

Wistar rats previously infected with rodent plasmodium berghei with a parasitemia of 20-30% was used as donor. Blood was collected by ocular puncture from the donor rats. The blood was diluted with physiological saline (0.9%) based on the parasitemia level of the donor rats to about 1×10^7 (16%). Malaria was induced by a single intraperitoneal dose and after four days parasitemia level was calculated and those rats with 19% parasitemia level or more were isolated for the experiment (Fidock *et al.*, 2004).

PARASITEMIA MEASUREMENT

Thin blood smears were made from the tail of each rat to determine the Percentage parasitemia of red blood cell. The smears will then be applied on microscope slides (76×26 mm), the slides were air-dried for a few minutes then fixed with absolute methanol, stained with 10% Giemsa stain allowed for 10 minutes, then washed gently using distilled water and dried at room temperature. Stained slides for each rat were examined under a microscope, with an oil (glycerol) immersion using $100 \times$ magnifications (Chessbrough, 2006; Schmidt, 2013). Ten different fields on each slide were examined to calculate the average parasitemia.

STATISTICAL ANALYSIS

All the results were analyzed using a statistical package for social science (SPSS version 25) and the results were expressed as mean \pm standard error of the mean (SEM). The data was statistically analyzed using one-way analysis of variance (ANOVA) with Tukey multiple comparison post hoc tests to determine the levels of significance between groups. Repeated measures (ANOVA) were done to determine the levels of significance between DAY7 and DAY21. P-Value ≤ 0.05 was considered statistically significant.

RESULTS

TABLE 2: EFFECT OF PARASITEMIA LEVEL FOR DAY 04

GROUP	MEAN \pm SEM (%)	P- VALUE	F- VALUE
NORMAL CONTROL	0.00 \pm 0.00		
EXPERIMENTAL CONTROL	29.33 \pm 0.33	0.00*	
ALLIUM SATIVUM	30.00 \pm 0.00	0.00*	
ZINGIBER OFFICINAL	26.00 \pm 0.58	0.00*	169.70
ALLIUM SATIVUM + ZINGIBER OFFICINAL	28.67 \pm 2.03	0.00*	
ARTEMETHER/LUMEFANTRINE	30.33 \pm 0.33	0.00*	
DIHYDROARTEMISINI AND PIPERAQUINE	29.00 \pm 0.58	0.00*	

* shows significant difference of $p < 0.05$ when compared with normal control.

Table 4.3 shows that parasitemia was significantly increased in test group 2 to 7 when compared with negative control (group 1).

TABLE 3: EFFECT OF PARASITEMIA LEVEL FOR DAY 08

GROUP	MEAN \pm SEM (%)	P- VALUE	F- VALUE
NORMAL CONTROL	0.00 \pm 0.00		
EXPERIMENTAL CONTROL	23.67 \pm 1.453	0.00*	
ALLIUM SATIVUM	9.33 \pm 0.667	0.00*	
ZINGIBER OFFICINAL	10.67 \pm 0.333	0.00*	109.778
ALLIUM SATIVUM + ZINGIBER OFFICINAL	12.33 \pm 0.667	0.00*	
ARTEMETHER/LUMEFANTRINE	7.33 \pm 0.333	0.00*	
DIHYDROARTEMISINI AND PIPERAQUINE	6.33 \pm 0.333	0.00*	

* shows significant difference of $p < 0.05$ when compared with normal control.

Table 4.4 shows that parasitemia was significantly increased in test group 2 to 7 when compared with negative control (group 1). It also shows that parasitemia was significantly reduced in group 3 to 7 when compared with positive control (group 2).

TABLE 4: EFFECT OF PARASITEMIA LEVEL FOR DAY 15

GROUP	MEAN \pm SEM (%)	P- VALUE	F- VALUE
NORMAL CONTROL	0.00 \pm 0.00		
EXPERIMENTAL CONTROL	30.00 \pm 1.732	0.00*	
ALLIUM SATIVUM	5.67 \pm 0.667	0.00*	
ZINGIBER OFFICINAL	7.67 \pm 3.333	0.00*	163.746
ALLIUM SATIVUM + ZINGIBER OFFICINAL	9.33 \pm 0.667	0.00*	
ARTEMETHER/LUMEFANTRINE	4.67 \pm 0.333	0.02*	
DIHYDROARTEMISINI AND PIPERAQUINE	2.67 \pm 0.333	0.61	

* shows significant difference of $p < 0.05$ when compared with normal control.

Table 4.5 shows that parasitemia was significantly increased in test group 2 to 6 when compared with negative control (group 1). It also shows that parasitemia was not significantly different between groups 1 and 7.

DISCUSSION

The effectiveness of any plant or drug on parasite clearance is estimated by its level of parasitemia (White, 2017). Higher level of parasitemia was established in the positive control group (group two) due to the multiplication effect of the parasite and they had the highest percentage parasitemia as also reported by (Basir *et al.*, 2012). While the various treatment groups had lower percentage parasitemia which suggested that garlic and ginger extracts maybe efficient in malaria parasites clearance. The parasitaemia suppressive effect of *A. sativum* can be said to be due to the presence of high concentration of allicin (Edeoga *et al.*, 2005) and thiosulfinates substance (Odugbemi *et al.*, 2007) while ginger was credited to phytochemicals like alkaloid (Coppi *et al.*, 2006).

CONCLUSION

Overall, the results confirm that administration of the different extracts were effective in parasite clearance especially single dose of allium sativum.

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